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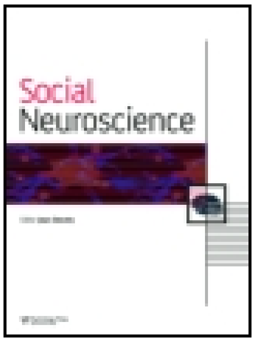

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Silja Sollberger, Thomas Bernauer, and Ulrike Ehlert*

*corresponding author

Affiliations:

Ulrike Ehlert, PhD
University of Zurich
Department of Psychology
Clinical Psychology and Psychotherapy
Binzmühlestrasse 14/26
CH-8050 Zurich, Switzerland
E-mail: u.ehlert@psychologie.uzh.ch
Phone: +41 44 635 7350; fax +41 44 635 7359

Silja Sollberger, MSc
University of Zurich
Department of Psychology
Clinical Psychology and Psychotherapy
Binzmühlestrasse 14/26
CH-8050 Zurich, Switzerland
E-mail: s.sollberger@psychologie.uzh.ch
Phone: +41 44 635 7358

Thomas Bernauer, PhD
ETH Zurich
Center for Comparative and International Studies (CIS) and
Institute for Science, Technology and Policy (ISTP)
IFW C.45.1
Haldeneggsteig 4
CH-8092 Zurich, Switzerland
E-mail: thbe0520@ethz.ch
Phone: +41 44 632 6771

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Abstract

Recently, cortisol has been suggested to moderate the positive relationship between testosterone and antisocial behavior. More precisely, high testosterone levels have been found to be related to aggressive or dominant behavior especially when cortisol levels were low. In the present study, we aimed to extend these findings to pro-environmental behavior as an indicator of prosocial behavior. In a first step, 147 male participants provided information on their everyday pro-environmental behavior by completing an online questionnaire on various energy-saving behaviors. In a second step, subjects provided two saliva samples for the assessment of testosterone and cortisol on two subsequent mornings after awakening. We found that testosterone was negatively related to pro-environmental behavior, but only in men with low cortisol. In conclusion, our findings provide first evidence for the joint association of testosterone and cortisol with everyday pro-environmental behavior. These results further reinforce the importance of considering interdependent hormone systems simultaneously rather than focusing on a single hormone.

Keywords: testosterone; cortisol; pro-environmental behavior; prosocial behavior; testosterone/cortisol ratio

Introduction

Individual differences in testosterone and cortisol concentrations have repeatedly been shown to be predictive of human social behavior. In the case of testosterone, previous research has mostly focused on aggression and dominance, with several reviews and meta-analyses providing evidence for a weak but positive relationship between testosterone and various forms of aggressive or antisocial behavior (Archer, 1991, 2006; Book, Starzyk, & Quinsey, 2001; Yildirim & Derksen, 2012). However, testosterone has also been investigated in the context of positive social behavior, such as cooperation, affiliation, empathy, and prosocial behavior. For example, inverse relationships have been found for testosterone and self-reported prosocial behavior (Harris, Rushton, Hampson, & Jackson, 1996), kindness, helpfulness, and warmth (Baucom, Besch, & Callahan, 1985), and smiling, pleasantness, and socially responsible behavior (Dabbs, 1997; Dabbs, Hargrove, & Heusel, 1996). Furthermore, testosterone has been shown to be negatively associated with affiliation and social bonding, as evident from lower testosterone concentrations in pair-bonded men and fathers as compared to single men and non-fathers (reviewed in Gray & Campbell, 2009). Testosterone also seems to be negatively related to cooperation motivation, as indicated by a finding that participants with high testosterone levels showed worse cognitive performance in a cooperative as compared to a competitive social environment (Mehta, Wuehrmann, & Josephs, 2009). Moreover, testosterone administration has been shown to reduce empathy (Hermans, Putman, & van Honk, 2006), collaborative decision-making (Wright et al., 2012), and generosity towards strangers (Zak et al., 2009). Notably, however, exogenous testosterone has also been reported to promote generosity (Eisenegger, Naef, Snozzi, Heinrichs, & Fehr, 2010) and reciprocity (Boksem et al., 2013) and to reduce lying (Wibral, Dohmen, Klingmüller, Weber, & Falk, 2012) in situations where the display of prosocial behavior is instrumental in gaining social status.

In contrast to testosterone, cortisol has repeatedly been found to be inversely related to aggressive and antisocial behavior. For example, lower cortisol has been shown to be associated with disruptive behavior disorder in boys (Dorn et al., 2009), callousness in male adolescents (Loney, Butler, Lima, Counts, & Eckel, 2006), as well as aggressive behavior (Böhnke, Bertsch, Kruk, & Naumann, 2010; van der Meij et al., 2015) and psychopathic traits (Cima, Smeets, & Jelicic, 2008; Holi, Auvinen-Lintunen, Lindberg, Tani, & Virkkunen, 2006) in adults. With regard to prosocial and related behaviors, evidence is scarce but positive associations have been reported for cortisol and the personality trait agreeableness (Tops, Boksem, Wester, Lorist, & Meijman, 2006; Vickers, Hervig, Poth, & Hackney, 1995), social affiliative behavior in children (Tennes, Kreye, Avitable, & Wells, 1986), quality of social relationships in adolescent girls (Booth, Granger, & Shirtcliff, 2008), and relationship functioning in mothers (Adam & Gunnar, 2001).

Apart from these partially opposing effects of testosterone and cortisol on social behavior, there is evidence for interdependent effects of the two steroid hormones on the biological level. Testosterone and cortisol are released by the hypothalamic-pituitary-gonadal (HPG) and the hypothalamic-pituitary-adrenal (HPA) axis, respectively, which have been shown to interact on multiple levels. Specifically, on the one hand, adrenal corticosteroids inhibit the HPG axis and thus reduce sex steroid release (Johnson, Kamilaris, Chrousos, & Gold, 1992; Mastorakos, Pavlatou, & Mizamtsidi, 2006; Rivier & Rivest, 1991), whereas testosterone, on the other hand, suppresses HPA axis function on the hypothalamic level (Viau, 2002).

Despite these well-known reciprocal effects on the biological level, it is only recently that researchers have started to more extensively examine interactive effects of testosterone and cortisol on the behavioral level. In this regard, the so-called *dual-hormone hypothesis* was recently proposed, which posits that cortisol might moderate testosterone's impact on status-seeking behavior (Carré & Mehta, 2011; Mehta & Josephs, 2010). In line with this, several studies have provided evidence for interaction effects of testosterone and cortisol on dominant and aggressive behavior (reviewed in Mehta & Prasad, 2015). For example, basal testosterone has been shown to be positively associated with aggression (Dabbs, Jurkovic, & Frady, 1991; Popma et al., 2007), dominance (Mehta & Josephs, 2010), testosterone rise in response to winning a competition (Zilioli & Watson, 2012), social status within a women's athletic team (Edwards & Casto, 2013), antisocial punishment (Pfattheicher, Landhäußer, & Keller, 2014), and risk-taking behavior (Mehta, Welker, Zilioli, & Carré, 2015) *exclusively* when cortisol levels were low. Conversely, in subjects with high cortisol the relationship between testosterone and aggressive or dominant behavior was non-existent (Dabbs et al., 1991; Edwards & Casto, 2013; Mehta et al., 2015; Pfattheicher et al., 2014; Popma et al., 2007), or even reversed (Mehta & Josephs, 2010). In addition, and of particular importance for the current hypotheses, one recent study found a reversed testosterone x cortisol interaction effect on self-reported empathy as a positive social behavior (Zilioli, Ponzi, Henry, & Maestripieri, 2014). Particularly, testosterone was negatively related to empathy among subjects with low cortisol, whereas this relationship was positive among individuals with high cortisol. Notably, however, these dual-hormone findings could not always be replicated and it has been argued that hormones and behaviors should be measured at approximately the same time for the effects to occur (Mazur & Booth, 2014). Furthermore, social context (Denson, Mehta, et al., 2013; Geniole et al., 2011) and personality traits (Tackett, Herzhoff, Paige, Page-Gould, & Josephs, 2014) have been suggested to moderate joint effects of testosterone and cortisol.

Statistically, these results were obtained by examining interaction effects of testosterone and cortisol on a given outcome variable by means of moderation analysis. However, an alternative method to investigate joint effects of testosterone and cortisol is to focus on the effects of the *ratio* of testosterone and cortisol (i.e., testosterone/cortisol). This index represents the level of testosterone relative to the level of cortisol within an individual and has been interpreted as an indicator of the general (im)balance between the HPA and HPG axes within an individual (Glenn, Raine, Schug, Gao, & Granger, 2011; Terburg, Morgan, & van Honk, 2009). Recently, and in line with the direction of the interaction effects reported in the existing literature, the testosterone/cortisol ratio has been proposed as a marker for social aggression (Terburg et al., 2009). More precisely, a higher ratio representing higher levels of testosterone relative to cortisol is hypothesized to predispose individuals towards more aggressive behavior. This supposition is based on the *triple balance model of emotion* (van Honk & Schutter, 2006) which was originally developed for the explanation of psychopathy and which suggests differential effects of testosterone and cortisol on the subcortical brain level. In particular, testosterone is argued to promote reward-seeking and approach behavior, whereas cortisol is proposed to increase punishment sensitivity and withdrawal behavior. Consequently, a combination of high testosterone and low cortisol levels is hypothesized to lead to a motivational stance of high reward versus low punishment sensitivity. This, in turn, makes individuals more likely to confront threat, which may result in more aggressive behavior (Terburg et al., 2009). In accordance with this hypothesis, a positive association between the ratio of testosterone (baseline) to cortisol (reactivity to a stressor) and psychopathy scores has recently been reported (Glenn et al., 2011). Furthermore, the testosterone/cortisol ratio has been shown to be related to marital violence (Romero-Martínez, González-Bono, Lila, & Moya-Albiol, 2013) and to predict neural activity in response to social threat (Hermans, Ramsey, & van Honk, 2008) and anger provocation (Denson, Ronay, von Hippel, & Schira, 2013). However, it is important to note that hormone ratios are associated with statistical concerns which must be addressed appropriately in the course of statistical analyses (Sollberger & Ehlert, in press).

Taken together, recent endocrine research on social behavior that considered testosterone and cortisol simultaneously has produced promising first results. Since these studies have mainly focused on aggressive and dominant behavior, we aimed to further investigate interaction effects of testosterone and cortisol with respect to prosocial behavior. We chose pro-environmental behavior as a specific form of prosocial behavior due to its high political and economic relevance, especially with respect to the issue of global climate change (Bernauer, 2013). Pro-environmental behavior encompasses various types of actions that are intended to minimize the individual's negative impact on the environment (e.g., minimize energy consumption, reduce waste production; Kollmuss & Agyeman, 2002; Steg & Vlek, 2009). Pro-environmental actions are prosocial by nature insofar as they typically do no entail direct benefits for the acting individual but rather serve long-term collective interests. In the environmental psychology literature, several models have therefore integrated pro-environmental behavior into the general framework of prosocial behavior (Bamberg & Möser, 2007; Kaiser & Byrka, 2011; Kollmuss & Agyeman, 2002; Stern, 2000).

Besides this, we investigated whether testosterone and cortisol are associated with pro-environmental awareness as a more *attitudinal* measure of environmentalism. Correlations between pro-environmental behaviors and attitudes are typically only moderate (Bamberg & Möser, 2007), which is in line with the well-known social psychological findings of inconsistent relationships between attitudes and related behaviors (e.g., Ajzen & Fishbein, 1977; Armitage & Christian, 2003). Notably, most of the studies described above reported endocrine influences on the extent of undesirable behavior such as violence, aggression, or dominance. However, there is one study in which interaction effects of testosterone and cortisol were investigated not only for a behavioral but also for a cognitive-emotional form of aggression (Popma et al., 2007). Interestingly, a testosterone x cortisol interaction effect was found only for the behavioral component. It might therefore be argued that endocrine factors are generally more strongly associated with behaviors than attitudes, possibly because of a more direct link which is less influenced by intervening or moderating variables. Accordingly, we expected the influence of testosterone and cortisol to be stronger on the behavioral than the attitudinal level.

In sum, the aim of the present study was to examine joint effects of testosterone and cortisol on pro-environmental behavior and awareness by focusing on interaction effects and effects of ratio scores. With regard to the interaction effect, we expected a negative relationship between testosterone and pro-environmental behavior, but only in individuals with low cortisol levels. Regarding the testosterone/cortisol ratio, we expected a negative effect of this ratio on pro-environmental behavior. In contrast, we assumed that for pro-environmental awareness, these endocrine effects would be weaker or even non-existent. Since the effect sizes of testosterone x cortisol interactions have proven to be small to medium, a sample size of 100 to 150 participants has recently been recommended to achieve sufficient statistical power (Carré & Mehta, 2011). Accordingly, we aimed for a large sample in the proposed range. Furthermore, previous studies investigating joint effects of testosterone and cortisol on antisocial behavior have mainly been conducted with very homogenous samples such as prison inmates (Dabbs et al., 1991), delinquent youths (Popma et al., 2007), or psychology students (Mehta & Josephs, 2010; Zilioli & Watson, 2012). Therefore, a further aim of the present study was to investigate effects of testosterone and cortisol in a more heterogeneous sample regarding age and occupational background.

Method

Participants

The final sample included 147 healthy male participants with a mean age of 36.5 years (*SD* = 14.7, range: 19-73). 40.8 % (*n* = 60) of the participants reported having completed education at the upper secondary level which corresponds to level 3 of the International Standard Classification of Education (ISCED; UNESCO, 1997). 57.8 % (*n* = 85) had reached the first stage of tertiary education (ISCED level 5) while 1.4 % (*n* = 2) did not report their education level. All subjects were part of a larger sample of men (*n* = 487) participating in an online survey on psychological factors associated with pro-environmental behavior. Participants were recruited from Germany, Austria, and the German-speaking part of Switzerland through online advertisements, mailing lists of different organizations, social networks as well as flyers displayed at different universities, train stations, shopping malls, and fitness centers. The study protocol was approved by the local ethics committee of the Faculty of Arts, University of Zurich, and all subjects provided written informed consent.

Procedure

Data were collected in two stages. In a first step, participants completed the above-mentioned online survey which included socio-demographic items and standardized questionnaires on everyday pro-environmental behavior, general pro-environmental awareness as well as chronic stress which was used as a covariate. Furthermore, the survey contained measures of personality and emotion regulation which are not reported here. Of a total of 883 subjects who started answering the online questionnaire, 487 (55.2 %; original sample) completed the whole survey. At the end of the questionnaire, subjects were asked whether they were interested in participating in the second part of the study in which two saliva samples were collected for the assessment of baseline testosterone and cortisol.

The interested participants (*n* = 332, 68.2 % of the original sample) received saliva sampling materials and detailed written instructions by mail. On two subsequent workdays, they collected one saliva sample at home after awakening. Self-sampling of saliva has been described as an adequate method for field studies (Garde & Hansen, 2005; Jensen, Hansen, Abrahamsson, & Nørgaard, 2011). It is widely used since it allows participants to collect samples non-invasively in their natural environment directly after awakening (see e.g., Chida & Steptoe, 2009; Gettler, McDade, & Kuzawa, 2011; Perini, Ditzen, Fischbacher, & Ehlert, 2012). To verify compliance with the detailed sampling instructions, subjects filled out a protocol of their activities during the 12 hours prior to saliva sampling (see below). They further provided general health-related information and written informed consent. On the second day, they mailed the two saliva samples, protocols of activities, questionnaire and consent form back to our lab in a stamped envelope. Saliva samples were received from a total of 229 participants (47.0 % of the original sample and 69.0 % of the subjects who received sampling materials). Back at the laboratory, the samples were immediately stored at -20°C. The time interval between collecting and freezing of the samples was on average 3.81 (*SD* = 1.93) days for the first and 2.76 days (*SD* = 1.88) for the second sample. Neither testosterone nor cortisol was correlated with the time interval between sampling and freezing (*p*'s > .25). In line with this, previous studies have shown that saliva samples can be mailed and/or stored at room temperature for at least 7 days and sometimes up to 4 weeks without cortisol or testosterone levels being affected (Clements & Parker, 1998; Dabbs, 1991; Garde & Hansen, 2005; Gröschl, Wagner, Rauh, & Dörr, 2001; Jensen et al., 2011; Kirschbaum & Hellhammer, 1989, 1994).

To increase external validity of the initial online survey, we aimed for a sample as diverse as possible with respect to variables such as age, health, education, and income. We therefore did not employ any inclusion or exclusion criteria aside from the requirement of male gender. Since the prospect of an individual feedback on testosterone and cortisol levels was used as an incentive for participants to complete the online survey, all interested subjects were allowed to participate in the second part of the study. However, since endocrine parameters are very sensitive to influences of medication as well as medical and psychiatric illnesses, samples used for hormonal assessments are usually carefully selected with respect to health-related parameters to include mostly young and healthy participants. Therefore, for our analyses we excluded all participants who reported currently taking medication and/or suffering from a self-reported medical or psychiatric illness requiring treatment (*n* = 82; 35.8 % in total). Excluded participants were significantly older than included participants (*t* (227)

= -6.85, $p < .001$), had significantly lower testosterone levels (t (227) = 2.87, $p = .004$), and higher scores of pro-environmental behavior (t (227) = -2.27, $p = .024$) and pro-environmental awareness (t (227) = -1.99, $p = .048$). The two groups did not differ with regard to chronic stress (t (227) = -0.50, $p = .617$) and cortisol levels (t (227) = 0.80, $p = .426$). Figure 1 provides an overview of the sample selection process.

– Please insert Figure 1 about here –

Pro-environmental behavior and awareness

Pro-environmental behavior and awareness were assessed with 6 subscales of a standardized questionnaire on energy consumption developed by Sütterlin et al. (2011). We used three scales to measure everyday energy-saving behavior in the housing domain (12 items; e.g., "*Turn off standby on appliances*"), mobility domain (4 items; e.g., *"Go on holidays by train"*), and food domain (4 items; e.g., *"Avoid buying foods from distant countries"*). Participants indicated on a six-point Likert scale how often they performed the described behaviors, with response options including *1 (never)*, *2 (rarely), 3 (once in a while), 4 (often), 5 (almost always)*, and *6 (always)*. There was also an option *does not apply to me*. To obtain a more general measure of pro-environmental behavior, we computed a composite behavioral score by averaging the three scale means, with higher scores indicating more pro-environmental behavior. The Cronbach's alpha computed based on these three means was satisfactory ($\alpha = 0.63$).

In addition to actual behaviors, we intended to measure subjects' general pro-environmental awareness in the area of energy conservation. Therefore, we used three more scales to measure general energy-related attitudes (4 items; e.g., *"Energy conservation is important to me"*), personal norms (4 items; e.g., *"I feel personally obliged to avoid unnecessary energy consumption wherever possible.")*, and motives of energy consciousness (3 items; e.g., *"I primarily pay attention to energy consumption in the household because of general energy consciousness."*). For these three scales, subjects indicated how much the presented statements applied to them on a scale from *1 (applies not at all)* to *6 (completely applies)*. As described for the behavioral measures, we computed a composite attitudinal score by averaging the three scale means (Cronbach's $\alpha = 0.76$), with higher scores indicating stronger pro-environmental awareness.

Chronic psychosocial stress

Chronic stress was included as a covariate since it is likely to be associated with hormonal status as well as pro-environmental behavior and awareness. We assessed chronic stress with the 12-item screening scale of the Trier Inventory for the Assessment of Chronic Stress (TICS; Schulz, Schlotz, & Becker, 2004). This scale includes items on chronic worrying, work overload, social overload, lack of social recognition, and excessive demands. Participants were asked to indicate the frequency of experience within the last 3 months for each item on a 5-point Likert scale ranging from *0 (never)*, over *1 (seldom)*, *2 (sometimes)*, and *3 (often)* to *4 (very often)*. Items were summed, with higher scores indicating higher levels of perceived chronic stress (minimum: 0, maximum: 48).

Testosterone and cortisol

The two saliva samples were collected using a commercially available sampling device employing the passive drool method (SaliCaps, IBL International GmbH, Hamburg, Germany). Participants were asked to provide approximately 1 ml of saliva on both sampling occasions. To minimize the confounding influence of external factors, subjects were asked to refrain from alcohol, caffeine, heavy exercise, and sexual activity 12 hours prior to the collection of saliva and to not eat, drink, smoke, chew gum, or brush their teeth 1 hour before sampling. Testosterone and cortisol were assessed from both samples with standard luminescence immunoassays using kits from IBL International GmbH (Hamburg, Germany). The samples were assayed in singlet at the biochemical laboratory at the Institute of Psychology of the University of Zurich. For testosterone, intra- and inter-assay coefficients were below 5% and 10%, respectively, while sensitivity was at 1.8 pg/ml. For cortisol, intra- and inter-assay coefficients were both below 5%, with a sensitivity of 0.003 µg/dL. Testosterone measured on day 1 was highly correlated with testosterone measured on day 2 ($r = .71$, $p < .001$), while the correlation between the two cortisol measurements was smaller but still highly significant ($r = .33$, $p < .001$). Thus, for all further analyses, the two values were averaged for each hormone to obtain more reliable measures of basal testosterone and cortisol. In the case of seven participants, one saliva container was damaged during the mailing process, rendering the assessment of testosterone and cortisol impossible. For these subjects, the single testosterone and cortisol values were entered into the analyses. We also repeated all analyses excluding these seven participants. This did not change the results. Therefore, all effects reported here are based on the data for all 147 subjects.

Protocol of activities

Since testosterone and cortisol are influenced by environmental factors, participants were asked to document their activities during the 12 hours prior to saliva collection on both sampling occasions. In particular, all subjects reported exact waking and collection times for each saliva sample as well as their usual waking time. Furthermore, they documented sleep duration, sleep disruptions and sleep quality, physical and sexual activity, alcohol and caffeine consumption, and smoking. They also provided more specific information on their activities and interaction partners during the last 12 hours.

Health-related information

To assess further possible confounders, subjects were asked to answer several health-related questions. They indicated their height and weight, smoking status, level of physical activity as well as frequency of alcohol consumption and drug use. Furthermore, they were asked whether they suffered from any physical diseases or mental disorders requiring treatment, and whether they were currently taking any medication.

Statistical analysis

We first computed zero-order correlations between all variables of interest (Spearman's rho, 2-tailed). Subsequently, we performed multiple linear regression analyses with either pro-environmental behavior or awareness as the dependent variable and testosterone, cortisol, and the testosterone x cortisol interaction as independent variables. Additionally, age and chronic stress were included as covariates. In the case of a significant interaction effect of testosterone and cortisol, we followed the procedure outlined by Aiken and West (1991) and performed additional simple slopes analyses. These allowed us to investigate the association between testosterone and pro-environmental behavior/awareness at a given level of cortisol. More precisely, we tested whether the relationship between testosterone and pro-environmental behavior/awareness was significantly different from zero when cortisol was set either one standard deviation above or below the mean.

In a complementary approach, we employed multiple indicators multiple causes (MIMIC) modeling, a special case of structural equation modeling, to test the same effects of age, chronic stress, testosterone, cortisol, and the testosterone x cortisol interaction on pro-environmental behavior and awareness. This method allowed us to include all three behavioral (or attitudinal) subscales into one model by forming a latent variable. In structural equation models with latent variables, measurement error is explicitly considered, which allows for a more correct estimation of the interesting relationships. Model fit was evaluated based on three different indices. First, we examined the χ^2 value as the traditional measure of model fit, with a non-significant result at the .05 threshold being indicative of a good model fit (Barrett, 2007). However, since the χ^2 is sensitive to sample size (Bentler & Bonett, 1980), we additionally inspected the Comparative Fit Index (CFI; Bentler, 1990), with values > .9 indicating a good fit (Bentler, 1992). Finally, we examined the root mean squared error of approximation (RMSEA), for which values < .05 represent a good and values < .08 a fair fit (Browne & Cudeck, 1992).

Furthermore, we computed the testosterone/cortisol ratio which has recently been proposed as a marker for social aggression (Terburg et al., 2009) by dividing testosterone levels by cortisol levels. The resulting ratio scores were log-transformed to account for the asymmetry problem of ratios (Sollberger & Ehlert, in press), and then used in multiple linear regression analyses along with age and chronic stress to predict pro-environmental behavior and awareness.

Structural equation modeling was performed using SPSS AMOS, version 22.0 (IBM, Armonk, NY), whereas all other analyses were conducted using SPSS, version 22.0 (IBM, Armonk, NY).

Results

Descriptive statistics and zero-order correlations

An overview of the descriptive statistics for all variables of interest is given in Table 1, whereas their zero-order correlations are shown in Table 2. In accordance with previous studies (Gettler et al., 2011; A. Gray, Jackson, & McKinlay, 1991; Mehta, Jones, & Josephs, 2008; Mehta & Josephs, 2010; Popma et al., 2007), testosterone and cortisol levels were modestly correlated ($r = .22$, $p = .008$). There was a positive association between pro-environmental behavior and awareness ($r = .65$, $p < .001$) but neither testosterone nor cortisol was correlated with the two pro-environmental measures. Pro-environmental behavior was positively associated with age ($r = .26$, $p = .001$) and correlated negatively with chronic stress ($r = -.24$, $p = .003$). Testosterone significantly decreased with age ($r = -.49$, $p < .001$) while cortisol was not associated with age ($r = -.09$, $p = .298$).

– Please insert Table 1 about here –

– Please insert Table 2 about here –

Interaction effects of testosterone and cortisol in relation to pro-environmental behavior

A hierarchical multiple regression analysis was conducted to predict pro-environmental behavior (see Table 3). The following variables were entered as predictors: age and chronic stress as covariates in step 1, testosterone and cortisol in step 2, and the testosterone x cortisol interaction in step 3. Overall, the final regression model was significant (F (5, 141) = 4.65, p < .001, R^2 = .142). Regarding the covariates, there were significant effects of age (β = .22, p = .012) and chronic stress (β = -.20, p = .014) on pro-environmental behavior. There were no significant main effects of testosterone (β = -.05, p = .577) and cortisol (β = .01, p = .882). However, in line with our hypothesis, a significant testosterone x cortisol interaction effect emerged (β = .20, p = .016, ΔR^2 = .036), indicating that the relationship between testosterone and pro-environmental behavior changes with varying cortisol levels, or, put differently, that the relationship between cortisol and pro-environmental behavior depends on testosterone.

We also performed the same analysis without including chronic stress as a covariate, which did not change the direction of the results (final model: F (4, 142) = 4.12, p = .003, R^2 = .104; age: β = .25, p = .004; testosterone: β = -.05, p = .542; cortisol: β = .02, p = .823; testosterone x cortisol: β = .17, p = .037, ΔR^2 = .028). When age, the second covariate, was additionally dropped from the analysis, the interaction effect failed to reach significance (final model: F (3, 143) = 2.57, p = .056, R^2 = .051; testosterone: β = -.16, p = .065; cortisol: β = .01, p = .877; testosterone x cortisol: β = .15, p = .070). However, it is important to note that age is an essential confounding variable in the current sample and should be considered as a covariate, since it ranged from 19 to 73 and was significantly associated with both testosterone (r = -.49, p < .001) and pro-environmental behavior (r = .26, p = .001).

– Please insert Table 3 about here –

To facilitate interpretation of the interaction, in Figure 2 predicted pro-environmental behavior scores are plotted at one SD above and below the means of testosterone and cortisol as outlined by Aiken and West (1991). Additional simple slopes analysis revealed a significant negative slope for the relationship between testosterone and pro-environmental behavior when cortisol equaled 1 SD below the mean (B = -0.22, t (141) = 2.13, p = .018). In contrast, when cortisol was set 1 SD above the mean, there was a positive but non-significant association between testosterone and pro-environmental behavior (B = 0.12, t (141) = 1.04, p = .151).

– Please insert Figure 2 about here –

In a further approach to visualize the interaction effect, we plotted the estimated marginal effect (beta value) of testosterone on pro-environmental behavior at different levels of cortisol. As shown in Figure 3, at the mean level of cortisol the relationship between testosterone and pro-environmental behavior was approaching zero, whereas it became more negative with decreasing cortisol levels, and more positive with increasing cortisol levels.

– Please insert Figure 3 about here –

Interaction effects of testosterone and cortisol in relation to pro-environmental awareness

Following the same procedure as outlined above for pro-environmental behavior, we investigated main and interaction effects of testosterone and cortisol on pro-environmental awareness, using age and chronic stress as covariates. However, neither the overall model (F (5, 141) = 0.91, p = .474, R^2 = .031) nor the effects of age (β = .14, p = .142), stress (β = -.09, p = .317), testosterone (β = .09, p = .337), cortisol (β = -.04, p = .635), or testosterone x cortisol (β = .10, p = .272) reached significance.

Structural equation models

In a complementary approach, we used a MIMIC model to predict the latent variable pro-environmental behavior (PEB) – consisting of the three indicators housing, mobility, and food – based on age, chronic stress, testosterone, cortisol, and the testosterone x cortisol interaction (see Figure 4). The model fitted the data fairly well (χ^2 (10) = 16.05, p = .098; CFI = .94; RMSEA = .064 [90%CI: .000-.120]), and latent pro-environmental behavior (R^2 = .227) was significantly associated with age (β = .29, p = .011), chronic stress (β = -.25, p = .019) as well as the interaction between testosterone and cortisol (β = .22, p = .033, ΔR^2 = .042). There were no significant main effects of testosterone (p = .555) or cortisol (p = .989).

– Please insert Figure 4 about here –

An analog MIMIC model was formulated to predict latent pro-environmental awareness – consisting of the three indicators energy-related attitudes, personal norms, and motives of energy consciousness – based on age, chronic stress, testosterone, cortisol, and the testosterone x cortisol interaction. The model fit was moderate (χ^2 (10) = 21.43, p = .018; CFI = .93; RMSEA = .088 [90%CI: .035-.140]), however, none of the predictors was significantly associated with latent pro-environmental awareness (age: $\beta = .15$, $p = .142$; stress: $\beta = -.13$, $p = .169$; testosterone: $\beta = .07$, $p = .506$; cortisol: $\beta = -.02$, $p = .861$; testosterone x cortisol: $\beta = .09$, $p = .356$).

Effects of the testosterone/cortisol ratio

We conducted further regression analyses with the testosterone/cortisol ratio as predictor and either pro-environmental behavior or awareness as dependent variable. Age and chronic stress were entered as covariates in step 1, while the log-transformed testosterone/cortisol ratio was entered in step 2. However, there were no significant effects of the testosterone/cortisol ratio with respect to either pro-environmental behavior ($\beta = -.04$, $p = .617$) or awareness ($\beta = .06$, $p = .531$).

Compliance with saliva sampling procedures

Self-reported compliance with the sampling procedures was high. Mean reported waking time was 7.00 am (*SD* = 1.33 hours), whereas the interval between awakening and sample collection was on average 12.71 min (*SD* = 9.64). Previous studies have shown that delays of up to 15 min between waking and sampling do not significantly affect waking cortisol levels (Dockray, Bhattacharyya, Molloy, & Steptoe, 2008; Kudielka, Broderick, & Kirschbaum, 2003). Accordingly, subjects are typically considered to be compliant if they provide the saliva sample within a 15 min window after awakening (Broderick, Arnold, Kudielka, & Kirschbaum, 2004; Holt-Lunstad & Steffen, 2007; Jacobs et al., 2005). The majority of the subjects indicated that they had refrained from sexual (98.64 %) and physical (77.55 %) activity, alcohol (89.80 %) and caffeine (90.48 %) consumption, and smoking (87.76 %) during the 12 hours before saliva collection on both sampling occasions. Nevertheless, to ensure that our results were not biased by systematic sampling errors, we repeated all analyses including the above mentioned variables as well as sleep duration and sleep quality as covariates. We further examined whether excluding non-compliant subjects had an effect on the analyses. Both procedures did not change our results. Therefore, the simplest models including all 147 participants are reported here.

Discussion

In the current study, the association between testosterone, cortisol, pro-environmental behavior, and pro-environmental awareness was investigated in a large male sample. The results showed a significant interaction effect of testosterone and cortisol on pro-environmental behavior, with a negative relationship between testosterone and pro-environmental behavior exclusively in subjects with low cortisol. For pro-environmental awareness, no such effect emerged.

Our results are consistent with a series of studies reporting moderating effects of cortisol on the relationship between testosterone and social behavior. In several studies, high testosterone was positively associated with indicators of aggressive or dominant behavior only in subjects with low cortisol (Dabbs et al., 1991; Edwards & Casto, 2013; Mehta & Josephs, 2010; Pfattheicher et al., 2014; Popma et al., 2007). Furthermore, one recent study found a negative relationship between testosterone and self-reported empathy exclusively among individuals with low cortisol. In line with the direction of these effects, the present study shows for the first time that in individuals with low cortisol, high testosterone is additionally associated with less pro-environmental behavior.

The mechanisms by which testosterone and cortisol influence social behavior are not yet fully understood. According to the triple balance model of emotion (van Honk & Schutter, 2006), testosterone and cortisol have opposing effects on the neurobiological and psychological level, with testosterone promoting reward-seeking and approach behavior, and cortisol increasing punishment sensitivity and withdrawal behavior. Accordingly, it has been argued that cortisol might inhibit the effects of testosterone on behavior through associated psychological variables such as social withdrawal and inhibition (Dabbs et al., 1991; Mehta & Josephs, 2010; Popma et al., 2007). In particular, while low cortisol, which is associated with low punishment sensitivity and inhibition, may allow for the full expression of dominant and aggressive behaviors associated with high testosterone, the behavioral correlates of high cortisol, on the other hand, might override the aggression-promoting effects of testosterone.

Interestingly, there is evidence indicating that support of environmental policies is negatively correlated with social dominance (Pratto, Sidanius, Stallworth, & Malle, 1994). It is therefore possible that dominance serves as a potential mediator of the relationship between testosterone, cortisol, and pro-environmental behavior. Future studies might thus benefit from including measures of dominance in order to test for direct versus dominance-mediated effects of testosterone and cortisol on pro-environmental behavior or other indicators of prosocial behavior. A further putative mechanism for effects of testosterone and cortisol on pro-environmental behavior involves the preference for immediate versus delayed rewards. Particularly, environmental protection requires that individuals weigh their future needs and well-being (e.g., future availability of essential natural resources) more heavily than their immediate needs (e.g., save time, money, or inconvenience by acting unsustainably) (cf. van

Vugt, Griskevicius, & Schultz, 2014). Generally, the extent to which people prefer present versus future rewards is considered to be an indicator of impulsivity and has been found to be associated with risk-taking behavior (Reynolds, 2006; Romer, Duckworth, Sznitman, & Park, 2010). Risk-taking, in turn, has recently been reported to depend on interactive effects of testosterone and cortisol (Mehta et al., 2015). Furthermore, as mentioned above, testosterone and cortisol exert opposite effects on reward sensitivity (van Honk & Schutter, 2006). Thus, testosterone and cortisol might jointly influence pro-environmental behavior through mediating reward-related preferences.

In the present study, the testosterone/cortisol ratio as suggested by Terburg et al. (2009) was not significantly associated with pro-environmental behavior. This is in accordance with previous studies that found significant testosterone x cortisol interaction effects but no effect of the testosterone/cortisol ratio in relation to dominance (Mehta & Josephs, 2010) and antisocial punishment (Pfattheicher et al., 2014). However, a few studies have also reported an effect of the testosterone/cortisol ratio, but no interaction effect (Glenn et al., 2011), or provided evidence for both, depending on the outcome measure (Denson, Ronay, et al., 2013). Yet other studies did not examine both types of effects, but focused exclusively on either interactions (Dabbs et al., 1991; Edwards & Casto, 2013; Popma et al., 2007; Tackett et al., 2014) or ratios (Hermans et al., 2008). Also, the statistical properties of ratios have often not been taken into account appropriately, which may have biased the results in some cases (cf. Sollberger & Ehlert, in press). Taken together, the existing evidence on the explanatory power of testosterone x cortisol interaction effects versus testosterone/cortisol ratios remains inconclusive. However, taking a closer look at the pattern of the current interaction effect (see Figure 2), the non-existent association between the testosterone/cortisol ratio and pro-environmental behavior in the present study is not surprising. Generally speaking, the overall pattern suggests that individuals with either high levels of both testosterone and cortisol, or low levels of both hormones, tended to act more pro-environmentally than individuals with high levels on one and low levels on the other hormone. Transferred to the ratio terminology, not only a comparably high testosterone/cortisol ratio (high testosterone combined with low cortisol) but also a comparably low ratio (low testosterone combined with high cortisol) tended to be less favorable than an intermediate ratio (low testosterone combined with low cortisol, or high testosterone combined with high cortisol) with respect to pro-environmental behavior. Therefore, it is not surprising that we did not find evidence for a linear relationship between the testosterone/cortisol ratio and pro-environmental behavior. In light of the present findings, it might rather be argued that individuals are most likely to behave pro-socially when there is a certain balance between their HPG and HPA axes activity, with neither testosterone nor cortisol predominating too strongly. As described above, an imbalance in favor of the HPG axis as indicated by a high testosterone/cortisol ratio has been suggested to be a marker for social aggression (Terburg et al., 2009; van Honk & Schutter, 2006). As far as we know, the reverse case, that is, an imbalance in favor of the HPA axis, has not to date been discussed in the context of social behavior. However, in the healthcare domain, a high cortisol/testosterone ratio has been reported to predict ischemic heart disease (Smith et al., 2005) and hospitalization related to congestive heart failure (Pereg et al., 2013), generally suggesting that higher cortisol relative to testosterone might be associated with lower cardiovascular health. In sum, further research is needed to answer the question whether it is possible to identify combinations of testosterone and cortisol concentrations which are particularly favorable with regard to prosocial behavior.

Interestingly, testosterone and cortisol were not associated with pro-environmental awareness as a more attitudinal measure of environmentalism. This is in line with the results of Popma et al. (2007) who reported that testosterone and cortisol were associated with a behavioral form of aggression but not with a cognitive-emotional form. Taken together, these findings suggest that effects of testosterone and cortisol manifest more strongly on the behavioral rather than on a cognitive/emotional level. This reinforces the notion that people's attitudes and actions are two different things (e.g., Ajzen & Fishbein, 1977; Armitage & Christian, 2003). Possibly, the link between endocrine factors and attitudes is weaker because it is more strongly influenced by intervening and moderating variables. However, further studies are needed to investigate whether this holds true for different behaviors and attitudes.

In the present study, testosterone and cortisol were measured in a large and diverse sample in an economic and participant-friendly way. Nevertheless, there are some limitations that need to be considered. First, participants collected both saliva samples at home and were therefore not supervised during sampling. However, compliance with the sampling procedures was high and we collected and averaged two saliva samples on two separate days in an effort to increase the reliability of the hormone assessment. Still, the correlation between the two cortisol measurements was only moderate, which might partially be attributable to the fact that cortisol concentrations change rapidly during the first hour after awakening (cortisol awakening response) and are therefore very sensitive to time of sampling. Future studies should thus attempt to measure testosterone and cortisol several times a day over multiple days and use electronic monitoring devices (Broderick et al., 2004; Kudielka et al., 2003) to further improve the reliability of the hormone measurements. Second, pro-environmental behavior and awareness were assessed using self-report measures, which are susceptible to effects of social desirability. Although it has been demonstrated that effects of social desirability on self-reported pro-environmental behavior and awareness are weak at most (Milfont, 2009), a more direct and implicit measure of pro-environmental behavior might have provided additional insights. It would therefore be interesting to further investigate the association of testosterone and cortisol with pro-environmental behavior in a laboratory or field setting where it is possible to directly observe different indicators of pro-environmental behavior such as donating, recycling, or consumer behavior. Future research is also required to examine whether the present findings generalize to female samples and different measures of prosocial behavior. Finally, it must be noted that our findings do not allow us to draw conclusions regarding the direction of the relationship between testosterone, cortisol, and pro-environmental behavior. More precisely, on the basis of our correlative approach, it is not possible to determine whether testosterone and cortisol directly caused pro-environmental behavior. This question can only be answered by exogenously altering testosterone and cortisol levels and examining subsequent changes in behavior (e.g., Eisenegger et al., 2010; Hermans, Putman, & van Honk, 2006).

In conclusion, the current research provides first evidence for the joint association of salivary testosterone and cortisol with pro-environmental behavior. In a large male sample with a wide age range, testosterone was negatively associated with energy-saving behavior, but only in subjects with low cortisol. These effects were restricted to actual behavior and did not generalize to pro-environmental awareness as a more attitudinal form of environmentalism. The present results suggest complex reciprocal effects of testosterone and cortisol and raise the question whether there might exist an ideal balance of HPG and HPA axis activity with regard to social behavior. Above all, the current findings further reinforce the importance of considering interdependent hormone systems simultaneously rather than focusing on single hormones.

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Table 1. Descriptive statistics

	Mean	*SD*	Minimum	Maximum
Testosterone (pg/ml)	82.94	37.44	15.83	216.50
Cortisol (nmol/l)	15.58	6.07	3.23	36.60
Pro-environmental behavior	4.24	0.65	2.36	5.42
Pro-environmental attitudes	4.27	0.89	1.33	5.92
Age	36.50	14.70	19	73
Chronic stress	16.69	8.18	1	37

Table 2. Zero-order correlations

	1.	2.	3.	4.	5.
1. Testosterone					
2. Cortisol	.22**				
3. Pro-environmental behavior	-.15	.06			
4. Pro-environmental attitudes	.02	.03	.65***		
5. Age	-.49***	-.09	.26**	.14	
6. Chronic stress	.12	.02	-.24**	-.10	-.16

$^{**}p < .01$, $^{***}p < .001$

Table 3. Multiple linear regression model for the prediction of pro-environmental behavior (final model)

	B	*SE B*	*β*	*t*	*p*
Constant	4.134	0.195		21.15	.000
Age	0.010	0.004	.22	2.54	.012
Chronic stress	-0.016	0.006	-.20	-2.49	.014
Testosterone	-0.001	0.002	-.05	-.56	.577
Cortisol	0.001	0.009	.01	.15	.882
Testosterone x Cortisol	0.000	0.000	.20	2.43	.016

B = unstandardized beta, *SE B* = standard error of *B*, *β* = standardized beta

Figure 1. Overview of the sample selection process.

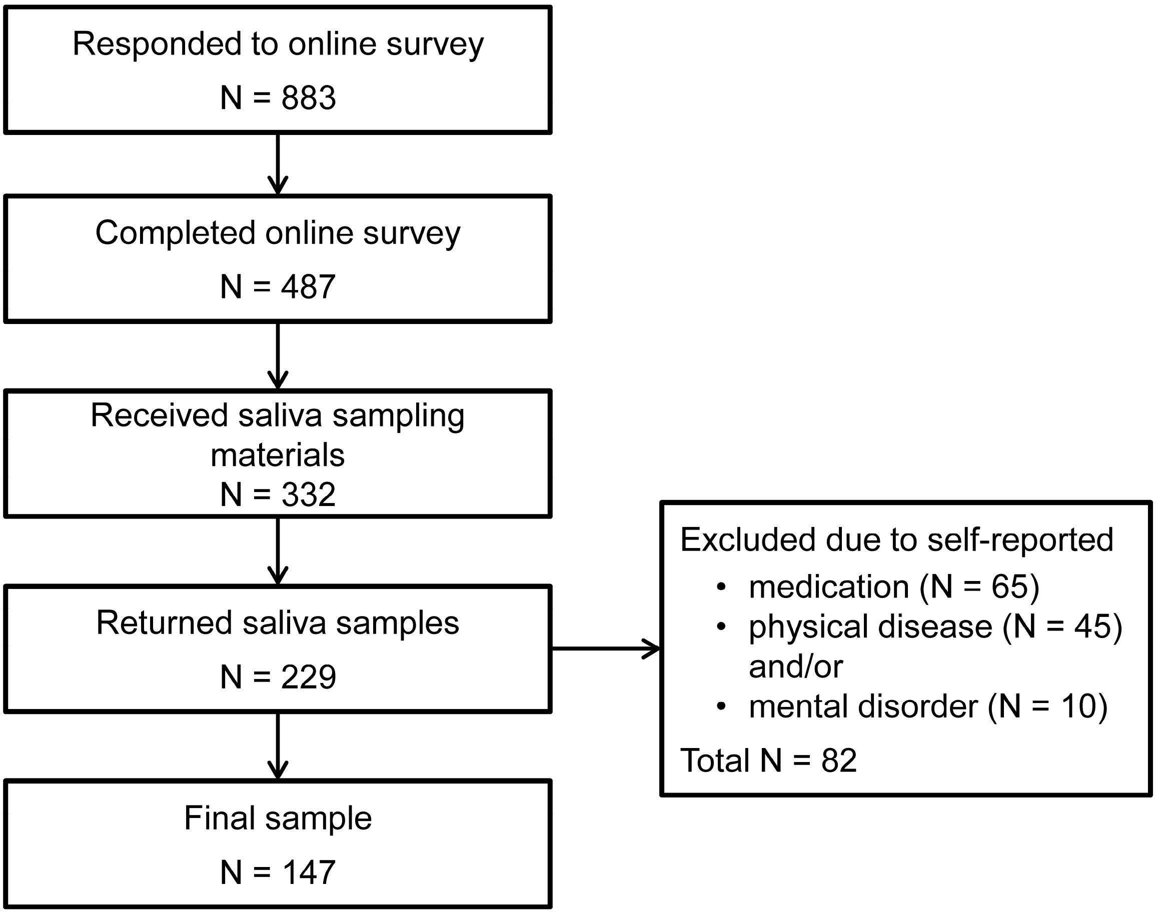

Figure 2. Interaction effect of testosterone and cortisol in relation to pro-environmental behaviour. Low: 1 *SD* below mean; high: 1 *SD* above mean.

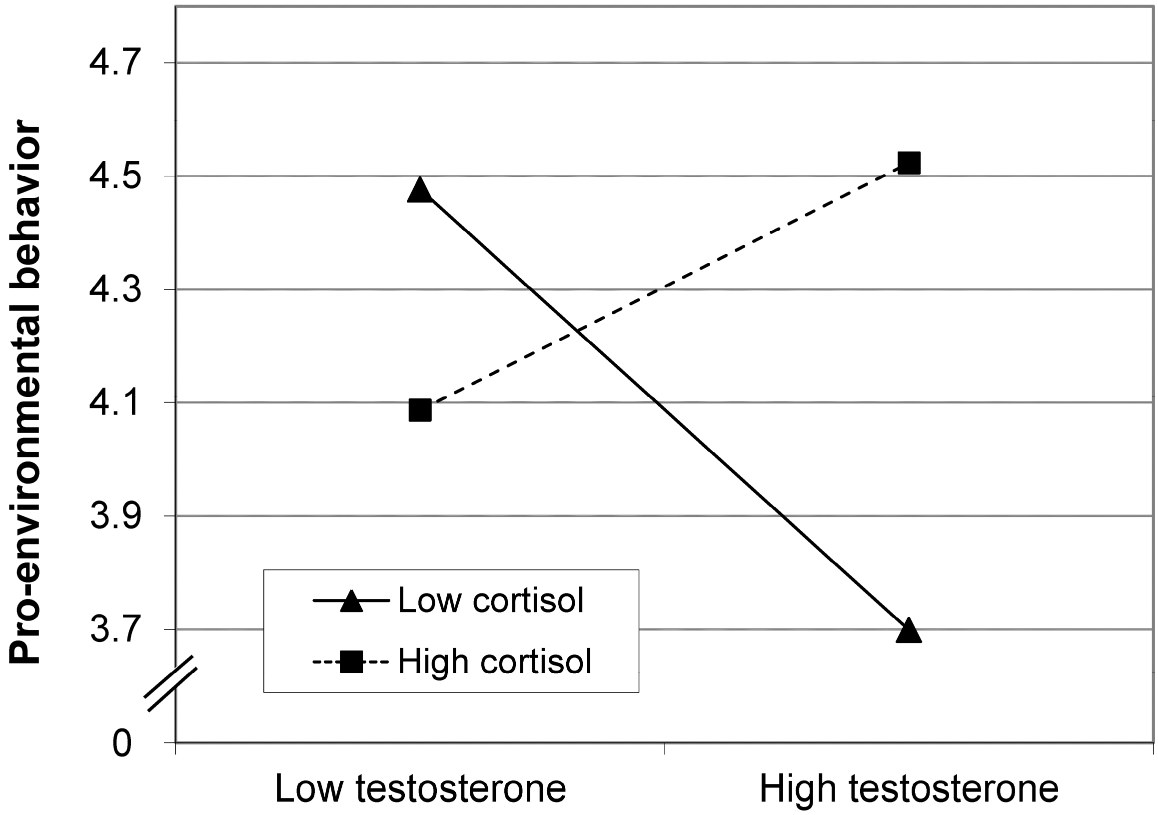

Figure 3. Marginal effect plot showing the relationship between testosterone and pro-environmental behaviour for different levels of cortisol. The dashed lines represent the 95% confidence interval, whereas the dotted vertical lines show the sample mean and *SD* of cortisol.

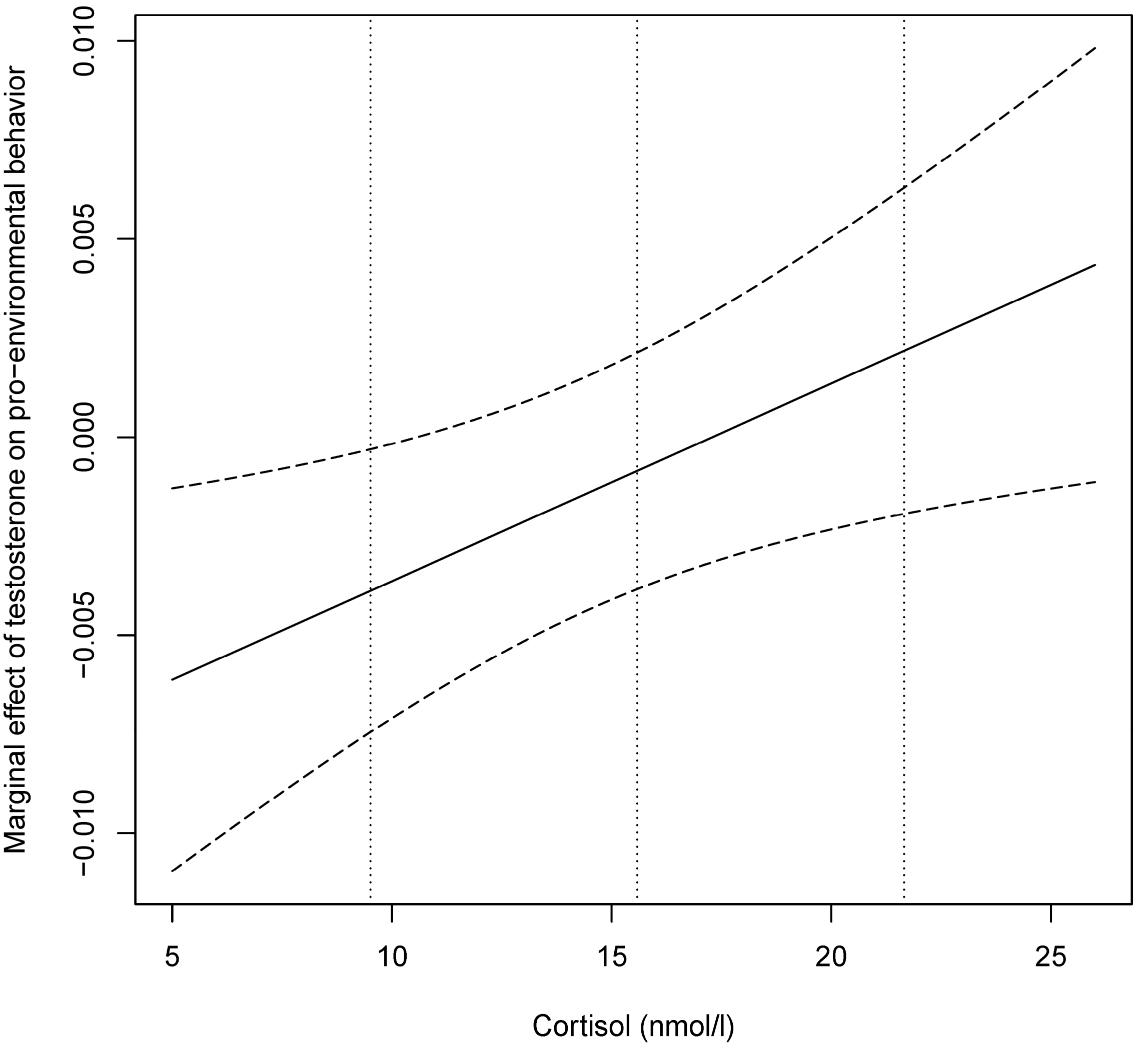

Figure 4. Multiple indicators multiple causes model for the prediction of the latent variable pro-environmental behavior (PEB). *p < .05, ***p < .001.